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(21) International Application Number: PCT/US94/06137 (22) International Filing Date: 31 May 1994 (31.05.94) (30) Priority Data: 08/073,234 7 June 1993 (07.06.93) US (71) Applicant: ADVANCED THERAPIES, INC. [US/US]; 330 Purissima Street, Half Moon Bay, CA 94019 (US). (72) Inventor: SCHREIER, Hans; 5335 Weber Road, Hermitage, TN 37076 (US). (74) Agents: SALIWANCHIK, David, R. et al.; Saliwanchik & Saliwanchik, Suite A-1, 2421 NW 41st Street, Gainesville, FL 32606-6669 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: LIPOSOME POWDERS (57) Abstract The subject invention concerns a unique procedure for producing dry liposome powders which can be formulated into a variety of pharmaceutical compositions. The process involves micronizing lyophilized liposome cakes.		

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DESCRIPTION

LIPOSOME POWDERS

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Background of the Invention

A liposome can be defined as any structure composed of lipid bilayers that enclose a volume. The lipid is not necessarily phospholipid but this is a commonly-used component. Liposomes can generally be formed by sonicating a lipid in an aqueous medium, by resuspension of dried lipid layers in a buffer, or by dialysis of lipids dissolved in a detergent solvent against a buffer of choice (New, R.R.C., Ed. [1990] *Liposomes: A Practical Approach*, Oxford University Press, New York).

Phospholipids form closed, fluid-filled spheres when they are mixed with water, in part because the molecules are amphipathic: they have a hydrophobic (water-insoluble) tail and a hydrophilic (water-soluble) or "polar" head. Two fatty acid chains, each containing from 10 to 24 carbon atoms, make up the hydrophobic tail of most naturally occurring phospholipid molecules. Phosphoric acid esters of choline, serine, glycerol, inositol, or other molecules compose the hydrophilic head. When a high enough concentration of phospholipids is mixed with water, the hydrophobic tails spontaneously align together to exclude water, whereas the hydrophilic heads bind to water.

The result is a bilayer in which the fatty acid tails point into the membrane's interior and the polar head groups point outward. The polar groups at one surface of the membrane point toward the liposome's interior and those at the other surface point toward the external environment. As a liposome forms, any water soluble molecules that have been added to the water are incorporated into the aqueous spaces in the interior of the spheres, whereas any lipid soluble molecules added to the solvent during vesicle formation are incorporated into the lipid bilayer.

When phospholipids are dispersed in an aqueous phase, a heterogeneous mixture of vesicular structures is usually formed, most of which contain multiple lipid bilayers forming concentric spherical shells. These were the liposomes first prepared and now called multilamellar vesicles (MLVs). If such a lipid dispersion is sonicated, the MLVs are reduced to much smaller structures in the size range 25-50 nm diameter.

These are called small unilamellar vesicles (SUVs) since they contain only a single bilayer. More recently, vesicles in the size range 100-500 nm diameter have been produced. These are called large unilamellar vesicles (LUVs).

5 Recently, liposomes have been studied extensively as a method for delivering drugs or other materials or compounds. In this regard, the desired drug can be encapsulated within the liposome by dissolving the drug in the water solution in which the liposomes are made. Liposomes employed for drug delivery typically range in diameter from 25 nm to several microns (for comparison, the diameter of an erythrocyte is about 10 microns) and are usually suspended in a solution.

10 Because of the nature of the lipid bilayer which forms the shell of the spherical liposome, liposomes present some difficulties in storage and formulation. Liposomes have been lyophilized (freeze-dried) in order to improve their physical stability, retention of encapsulated material, and overall shelf-life (Vanlerberghe/L'Oreal BE 873865, 8/1/79; Schneider/Batelle GB 200,2319; 2/24/82; Janoff/Liposome Comp. IL 15 76010, 12/31/85; Crowe/Regents Univ. Cal., WO 86/03938, 7/16/86; Moro/Farmitalia, U.S. Patent No. 4,746,516, 5/35/88; Schmidt/Vestar WO/90/03795; Ozer, Y. *et al.* [1988] *Acta Pharm. Technol.* 34:129-139; Ausborn, M. *et al.* [1992] *Eur. J. Pharmaceut. Biopharm.* 38:133-139). Such lyophilization procedures are well known and readily practiced by those skilled in the art and the process of lyophilization, 20 cryoprotection, and dehydroprotection, respectively, of liposomes, has been investigated extensively over the last several years (Talsma, H., M.J. van Steenbergen, P.J.M. Salemink, D.J.A. Crommelin [1991] *Pharm. Res.* 8:102-106; Talsma, H., M.J. van Steenbergen, D.J.A. Crommelin [1992] *Cryobiology* 29:80-86; Talsma, H., M.H. van Steenbergen, D.J.A. Crommelin [1991] *Int. J. Pharmaceut.* 77:119-126; see also 25 Özer, Y., H. Talsma, D.J.A. Crommelin, A. Hincal [1988] *Acta Pharm. Technol.* 34:129-139; Ausborn, M., P. Nuhn, H. Schreier [1992] *Eur. J. Pharmaceut. Biopharm.* 38:133-139).

30 Lyophilization results in the formation of a porous cake which has a large surface area and which can be rapidly and readily reconstituted prior to use to yield an aqueous dosage form. This aqueous dosage form can then be used for intravenous injection or other such routes of administration.

While lyophilization is the method of choice to preserve liposomes for future reconstitution to an aqueous form, lyophilized cakes cannot directly be incorporated in other pharmaceutical dosage forms. Specifically, there currently is no convenient method for preparing liposomes for administration in a dry or semi-solid state. Such dry or semi-solid states include powders filled in gelatine capsules or compressed powder mixtures in the form of tablets which can be administered orally. Other dry or semi-solid formulations which would be desirable for liposomes include powders to be sprinkled on healthy or diseased skin for cosmetic or therapeutic purposes; powders suspended in cremes, ointments, pastes, or lotions for use on healthy or diseased skin or mucosal membranes (e.g., buccal or vaginal membranes) for cosmetic or therapeutic purposes; and powders suspended in waxy bases, e.g., carbopols, to form suppositories for rectal or vaginal application.

Among the many potential uses of liposomes is inhalation therapy wherein medicaments enclosed within a liposome are delivered by inhalation. The use of liposome aerosols for inhalation therapy has been studied experimentally in mice (Myers, M.A., D.A. Thomas, L. Straub, D.W. Soucy, R.W. Niven, M. Kaltenback, C.I. Hood, H. Schreier, R.J. Gonzalez-Rothi [1993] *Exp. Lung Res.* 19:1-19), sheep (Schreier, H., K.J. McNicol, M. Ausborn, D.W. Soucy, H. Derendorf, A.A. Stecenko, R.J. Gonzalez-Rothi [1992] *Int. J. Pharmaceut.* 87:183-193), and human volunteers (Thomas, D.A., M.A. Myers, B.M. Wichert, H. Schreier, R.J. Gonzalez-Rothi [1991] *Chest* 99:1268-1270; see also Schreier, H. [1992] *J. Liposome Res.* 2:145-184). Furthermore, the *in vitro* performance of liposome aerosols has been studied as a function of liposome lipid composition (Niven, R.W., H. Schreier [1990] *Pharm. Res.* 7:1127-1133), size (Niven, R.W., M. Speer, H. Schreier [1991] *Pharm. Res.* 8:217-221), and operating conditions (Niven, R.W., M.T. Carvajal, H. Schreier [1991] *Pharm. Res.* 9:515-520). The liposome composition and procedures which have been used to date for liposome inhalation studies involve the use of nebulizers to produce the liposome particles, and these procedures have drawbacks which limit the utility of these compositions and methods.

Brief Summary of the Invention

The subject invention provides, for the first time, liposomes in a free-flowing dry powder form. This powder form is highly advantageous because it enables the use of liposomes as a method of delivering drugs and other compounds in formulations such as pills, cremes, gels, and powders which have not previously been possible. Specifically, the subject invention concerns the unexpected finding that lyophilized liposome cakes survive further processing to form advantageous free-flowing liposome powders. This further processing can be done with an appropriate milling device to produce particles in a size range which produces a free-flowing dry powder.

In a preferred embodiment of the subject invention, lyophilized liposome cakes are processed with a jet mill. Jet mills and their use are generally well known to those skilled in the art. However, jet mills and the like have not previously been used with lyophilized liposome cakes. When the procedures of the subject invention are utilized, milling results in the formation of micronized liposome particles in a size range of about 1 to about 100 μm diameter. In one embodiment, aggregation of such micronized powders is inhibited by the use of electrically charged lipid mixtures. This can be accomplished by, for example, incorporation of the negatively charged phosphatidylglycerol into the lipid mixture used to form the liposome. The procedures of the subject invention create flocs of weakly bound particles which are separated upon delivery of the dosage form.

The flow properties of the liposome powders of the subject invention can further be improved by mixing the liposome powder with carrier powders such as spray-dried lactose and other carbohydrates. The liposome powders may also be mixed with carbohydrate alcohols such as mannitol or sorbitol, with cellulose (e.g., Avicell), or with silica derivatives with a size range of, for example, 40-100 μm diameter.

Detailed Disclosure of the Invention

The subject invention pertains to a method for making a dry, free-flowing liposome powder. In a preferred embodiment, this liposome powder comprises phospholipids and can be in combination with other lipidic material, or with a variety of other compounds. The liposome powders of the subject invention form a free-

flowing dry powder and advantageously retain the original vesicular structure of the liposomes. Reference herein to "dry" powders refers to powders which are essentially free of water. The liposome components of these powders are in particles which range in size from about 1 μm to about 100 μm . These liposome particles are weakly bound flocs obtained by micronizing lyophilized liposome cakes. The particle flocs can comprise individual liposomes which are typically around 0.2 μm in diameter. The particle flocs typically comprise additional material which has been added as a cryoprotectant or bulking agent before or during the lyophilization process. Such additional material may be lactose, for example. The micronized liposome floc particles can then be dispersed in a carrier powder which typically consists of particles of similar or somewhat greater size compared to the liposome particles.

Thus, in a preferred embodiment, the subject invention concerns a process to micronize lyophilized liposome cakes with a jet mill, ball mill, or other manual or mechanical milling device to generate dry powder particles with a diameter of about 1 to about 100 μm . Preferably, these particles are from about 1 to about 10 μm . The process of the subject invention advantageously forms a stable flocculated micronized liposome powder formulated to form weakly bound flocs which are dispersed upon delivery of the dosage form.

In an initial step of the subject invention, liposomes can be formed utilizing any one of a number of procedures well known to those skilled in the art. Typically, this will involve introducing a lipid into an aqueous solution. A variety of other components can be added to create liposomes with particular desired characteristics. This is also the point at which compounds to be encapsulated can be dissolved into the aqueous solution. These water-soluble compounds would be enclosed in the inner core of the liposome which contains the aqueous solution. With lipid-soluble compounds, the compounds can be incorporated into the lipid bilayer of the liposome.

Lipids which can be used in making the liposomes include, but are not limited to, phospholipids including phosphatidylcholine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, cardiolipin, sphingomyelin, ceramides, cholesterol, dicetylphosphate, fatty acids, stearylamine, synthetic single-chain or double chain cationic, neutral, or anionic lipid constructs.

Once the liposomes are prepared, they are then lyophilized (freeze-dried) to produce a porous cake composition. To prepare the liposome for the lyophilization process, cryoprotectants can be added to the liposome solution. Such cryoprotectants and their use are well known to those skilled in the art. The cryoprotectants help to reduce the possibility of the liposome being damaged by the freezing process. In one embodiment, a carbohydrate can be added as the cryoprotectant during the liposome formation step of the process. In a preferred embodiment, a lipid to carbohydrate ratio of about 1:1 to about 3:1 can be used.

Lyophilization processes are well known to those skilled in the art and can be readily used to create dry, porous cake compositions comprising liposomes which encapsulate desired materials.

Once a lyophilized liposome cake is obtained, the next step of the process of the subject invention involves micronizing this cake with an appropriate micronizing device. As used herein, "micronizing" refers to the process whereby particles of about 1 to about 100 μm are produced from a large clump or cake preparation. In a preferred embodiment, a jet mill or equivalent device produces micronized particles from the lyophilized liposome cake in a size range from about 1 μm to about 100 μm and, preferably, from about 1 μm to about 10 μm . These particles are essentially stable flocs which contain the intact liposomes which are typically embedded or otherwise associated with lactose or other cryopreservative or bulking agent. These weakly-bound flocs are dispersed upon delivery of the dosage forms.

Thus, if a carbohydrate or other equivalent has been added in the initial liposome-forming step, the liposome composition obtained after micronization will be dispersed in the a carbohydrate preparation. The micronized liposomes can then be further dispersed in carrier powder which can be used to modulate the free-flowing properties of the liposome powder. As used herein, "free-flowing" refers to a powder which consists of particles that move past each other freely without substantial chemical or physical interaction which could produce clumps or a sticky consistency. In this regard, a variety of carrier powders can be used which will give the desired flow characteristics to the liposome powder. The carrier powder can also be used essentially as a diluent to decrease the concentration of the liposomes dispersed in the powder.

Carrier powders useful according to the subject invention include, but are not limited to, carbohydrates including lactose, maltose, saccharose, and trehalose. Also, carbohydrate alcohols can be used, and these would include mannitol, sorbitol, and xylitol. Other carriers which can be used include cellulose derivatives and silica derivatives. Spray-dried lactose is particularly advantageous as a carrier powder because its spherical particulate characteristics enhance the free-flowing nature of the liposome powder. Thus, one composition of the subject invention comprises micronized liposome powders comprising liposomes and spray-dried lactose.

The micronized liposome powder of the subject invention can be formulated into a variety of useful products. For example, a micronized liposome powder can be formulated as an oral powder capsule. In one embodiment, the oral powder capsule may be coated with polymeric coats to impart pH-sensitivity for selective release in the gastrointestinal tract.

Also, the liposome powder of the subject invention can be mixed with commonly used tablet powder components and compressed to give an oral tablet. As with the oral powder capsules described above, these tablets may be coated with polymeric coats to impart pH-sensitivity for selective release in the gastrointestinal tract. Alternatively, the tablets may be formulated with effervescent materials for rapid dissolution in water prior to oral administration.

Also, a micronized liposome powder can be formulated with waxy materials and fats to form suppositories for rectal and vaginal application.

Additionally, the liposome powder can be formulated as a suspension in cremes, ointments, pastes, or lotions for dermal and mucosal application.

In a further embodiment, the liposome powder can be formulated as a powder for inhalation to be used with a dry powder inhaler for delivering drugs to the nose, mouth, trachea, and lungs. Thus, one embodiment of the subject invention provides an alternative approach of liposome aerosol stabilization. In this embodiment, spray-dried lactose is a particularly attractive carrier powder because of its free-flow characteristics.

Materials and Methods

Reagents. L- α -phosphatidylcholine [plant] (SPC; in ethanol) and 1-palmitoyl-2-oleyl phosphatidylglycerol [sodium salt] (POPG; in chloroform) were purchased from Avanti Polar Lipids, Alabaster, AL. Cholesterol (Chol; Sigma grade [99+%]), α -lactose (monohydrate, 2% β content, substantially glucose-free), and *tert*-butanol were from Sigma Chemical Co., St. Louis, MO. Spray-dried lactose DCL 11 Pharmatose was supplied by DMV Ridgeview, Inc., La Crosse, WI). 5,6-carboxyfluorescein (CF) was from Eastman Kodak, Rochester, NY, and was purified according to the method of Ralston *et al.* (Ralston, E., I.M. Hjelmeland, R.D. Klausner, J.N. Weinstein, R. Blumenthal [1981] *Biochim. Biophys. Acta* 649:133-137). N,NQ-bis(1-hexylheptyl)-3,4:9,10-perylene-bis(dicarboximide) (BHPG; dissolved in methanol/chloroform 1:1 vol/vol to give 1 mg/ml) was a generous gift of Dr. R.A. Schwendener, University Hospital, Zurich, Switzerland.

Liposome preparation. Liposome formulations were prepared as follows: SPC/POPG/lactose and SPC/Chol/lactose, both at a molar ratio of 7:3, were prepared either with CF solution (\approx 296 mosm; water-soluble fluorescent marker), or with 0.006 mole parts BHPD (lipid-soluble fluorescent marker; added to the initial lipid mixture in organic solvent) in 10 mM phosphate buffer, pH = 7.5. All solutions used to prepare liposomes had a sufficient lactose concentration to achieve a 3 to 1 molar ratio of lactose to lipid (345 mM lactose; 115 mM total lipid).

Each lipid formulation was dried via rotary evaporation in a round-bottom flask followed by hydration in the appropriate CF- or buffer-lactose solution. Each preparation was extruded by passing at least 21 times through a 100 nm polycarbonate filter using a central filter housing connected to dual syringes (LiposoFast, Avestin Inc., Ottawa, Canada) as described by MacDonald *et al.* (MacDonald, R.C., R.I. MacDonald, B.P.M. Menco, K. Takeshita, N.K. Subbarao, L. Hu [1991] *Biochim. Biophys Acta* 1061:297-303).

Unencapsulated CF was removed via column chromatography (Sephadex G-75, swollen in lactose solution [125 mg/ml in phosphate buffered saline]).

Liposome lyophilization. The liposomes were lyophilized in an Edwards Model 12K Supermodulyo freeze-dryer (Edwards High Vacuum, West Sussex, England) according to the following protocol: the samples were shelf-frozen at \approx 0.7RC/minute to \approx -40RC, held at \approx -40RC for \approx 1.5 hours, then dried under vacuum

with 14.5 hours of primary drying at -35RC, followed by 6.5 hours of secondary drying at 25RC. Samples were stoppered under vacuum. Samples were stored at ambient temperature.

Jet milling. Lyophilized liposome samples were jet milled (Trost Impact Pulverizer, Gem T Research Model, Garlock Inc., Plastomer Products, Newtown, PA). Micronized powders were generated by the principle of opposing jets and cyclone separation. Samples were filled and the mill operated under dry nitrogen so as to minimize potential oxidation and absorption of trace amounts of water. Samples were milled for 3 minutes at an inlet pressure of 40 psig and 2 minutes at 50 psig with an opposing pressure of 50 psig in both cases. The majority of the sample was collected in the cyclone (5-10 μm particle size) rather the collection vessel (<5 μm), with a total recovery after milling of \approx 30-40%. During the milling process, there was no evidence of smearing.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 — Production of Liposome Powder

Nine grams of soy phosphatidylcholine (115 mM) were dispersed in 100 ml of an aqueous solution containing 8.6 grams of lactose (345 mM). Liposomes were extruded through 0.2 μm polycarbonate membranes under nitrogen pressure and then lyophilized according to a standard protocol. The lyophilized cake is scraped into a jet mill and the mill operated under dry nitrogen so as to minimize potential oxidation and absorption of water. Liposomes were milled for 3 minutes at an inlet pressure of 40 psig and 2 minutes at 50 psig with an opposing pressure of 50 psig. A majority of the mass introduced into the jet mill was collected in the cyclone of the mill representing a particle size of 5-10 μm diameter.

Example 2 — Use of Negative Surface Charge in Making Liposome Powders

6.3 grams of soy phosphatidylcholine were combined with 2.7 grams of phosphatidylglycerol to impart a net negative surface charge to the liposome particles. The liposomes were generated and processed to yield a dry liposome powder as in Example 1.

Example 3 — Liposome Powder Capsule

Liposome powder was introduced into gelatine capsules (Elanco HC #2) with the help of a manual capsule filling machine. Capsules were closed with gelatine tops and weighed to determine accuracy of filling. Capsules were dipped into Eudragit solution to impart a polymer coat for enteric coating.

Example 4 — Liposome Powder Inhalant

Ten milligrams dry powder were mixed with 10 mg spray-dried lactose and filled into a gelatine capsule. The gelatine capsule was introduced into a dry powder inhaler device (Spinhaler). The device was attached to an impactor device which measures aerosol particle size and size distribution. The capsule is perforated by the inhaler and air drawn through the device by a vacuum pump for 4 seconds. The powder aerosol generated was collected in the impactor and the size distribution determined using carboxyfluorescein as fluorescent marker encapsulated in the liposomes.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Claims

1 1. A free-flowing liposome powder, essentially free of water, comprising
2 micronized, lyophilized liposome particles.

1 2. The liposome powder, according to claim 1, wherein said micronized
2 liposome particles have a size between about 1 μm and about 100 μm .

1 3. The liposome powder, according to claim 2, wherein said micronized
2 liposome particles have a size between about 1 μm and about 10 μm .

1 4. The liposome powder, according to claim 1, which further comprises a
2 carrier powder.

1 5. The liposome powder, according to claim 4, wherein said carrier powder
2 is selected from the group consisting of carbohydrates, carbohydrate alcohols,
3 cellulose, and silica.

1 6. The liposome powder, according to claim 5, wherein said carrier powder
2 is spray-dried lactose.

1 7. A pharmaceutical composition comprising a liposome powder which
2 comprises micronized, lyophilized liposome particles.

1 8. The pharmaceutical composition, according to claim 7, wherein said
2 particles have a size between about 1 μm and about 10 μm .

1 9. The pharmaceutical composition, according to claim 6, which further
2 comprises a carrier powder.

1 10. The pharmaceutical composition, according to claim 9, wherein said carrier
2 powder is selected from the group consisting of carbohydrates, carbohydrate alcohols,
3 cellulose, and silica.

1 11. The pharmaceutical composition, according to claim 10, wherein said
2 carrier powder is spray-dried lactose.

1 12. The pharmaceutical composition, according to claim 7, wherein said
2 composition is in a form selected from the group consisting of pills, suppositories,
3 powders, gelatins, aerosols, ointments, pastes, and cremes.

1 13. A method for preparing a free-flowing liposome powder, essentially free
2 of water, wherein said method comprises the following steps:

3 (a) preparing an aqueous suspension of liposomes;

4 (b) lyophilizing said aqueous suspension of liposomes to give a porous
5 lyophilized liposome cake;

6 (c) micronizing said porous lyophilized liposome cake to produce liposome
7 particles having a size between about 1 μm and about 100 μm .

1 14. The method, according to claim 14, which further comprises adding a
2 carrier powder to said micronized liposomes.

1 15. The method, according to claim 14, wherein said carrier powder is selected
2 from the group consisting of carbohydrates, carbohydrate alcohols, cellulose, and
3 silica.

1 16. The method, according to claim 15, wherein said carrier powder is spray-
2 dried lactose.

1 17. The method, according to claim 13, wherein said micronization is
2 accomplished with a jet mill.

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1 18. The method, according to claim 13, which further comprises use of an
2 electrically-charged lipid mixture to form said liposomes in order to reduce
3 aggregation.

1 19. The method, according to claim 18, wherein said electrically-charged lipid
2 mixture comprises phosphatidylglycerol.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/06137

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 260 241 (AKTIEBOLAGET DRACO) 16 March 1988	1-16
Y	see the whole document ---	17
X	EP,A,0 170 642 (AKTIEBOLAGET DRACO) 5 February 1986 see page 17; example 2 ---	1-16
Y	WO,A,87 07502 (PHARES PHARMACEUTICAL RESEARCH N.V.) 17 December 1987 see page 18; example 12 ---	17
X	EP,A,0 152 379 (CIBA-GEIGY AG) 21 August 1985 see page 43 - page 44; example 11 ---	1-16
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 085 729 (DAINIPPON PHARMACEUTICAL CO.LTD.) 6 May 1982 see page 3 - page 4; example 2 -----	1-16, 18, 19

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
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